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Morphological evidence for field effect as a mechanism for tumour spread in mammary Paget's disease

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Aims: The histogenesis of mammary Paget's disease is controversial. The purpose of this study was to investigate the mechanism of tumour spread in the nipple epidermis by examining 28 cases of mammary Paget's disease associated with underlying intraductal carcinoma.
Methods and results: The atypical cells in the epidermis displayed a spectrum of cytological changes ranging from small-sized atypical cells located in the basal cell layer to large-sized atypical cells characteristic of Paget's cells in the upper layer of the epidermis. Serial sectioning revealed the presence of isolated, scattered and small atypical cells in the basal cell layer at the periphery of the epidermal lesion. The atypical cells, including those in the basal cell layer showed positive immunostaining for cytokeratin 7 and Her2/neu oncoprotein. Electron microscopy examination demonstrated the presence of intercellular junctions of desmosomal-like or desmosomal types between tumour cells and adjacent squamous cells. Furthermore, examination of the intraductal carcinoma of the breast tissue in cases of Paget's disease as well as control cases of intraductal carcinoma also revealed areas of skip lesions of intraductal carcinoma.
Conclusions: In view of these changes, it is unlikely that tumour expansion or tumour cell motility are sufficient explanations to account for the pattern of tumour spread in both the epidermis and the duct epithelium with skip lesions. A 'field effect' in the duct system harbouring intraductal carcinoma and the adjacent epidermis may play an important role in the tumour cell spread in the epidermis as well as in the ductal epithelium.

Keywords: breast, carcinoma, ductal carcinoma in situ, field effect, Paget's disease

Introduction

Paget's disease of the nipple is characterized by the presence of neoplastic cells in the epidermis and is most often associated with underlying ductal carcinoma in situ (DCIS). The intraepidermal neoplastic cells (or Paget's cells) and the DCIS cells are usually similar in both cytological appearance and immunohistochemical and oncogenic expression.[1–3] In addition, many histopathological and immunohistological features of mammary Paget's cells are similar to those seen in extramammary Paget's cells.[4–10] Despite this similarity, the pathogenesis of mammary Paget's disease is considered different from that of the extramammary sites. In the latter sites, the neoplastic cells arise from the epidermis, whereas those of the nipple are commonly believed to develop as a result of secondary spread due to the extension or migration of malignant cells from the underlying breast carcinoma.[7,11,12]

In this study, the histopathological and immunohistochemical features of the disease and underlying

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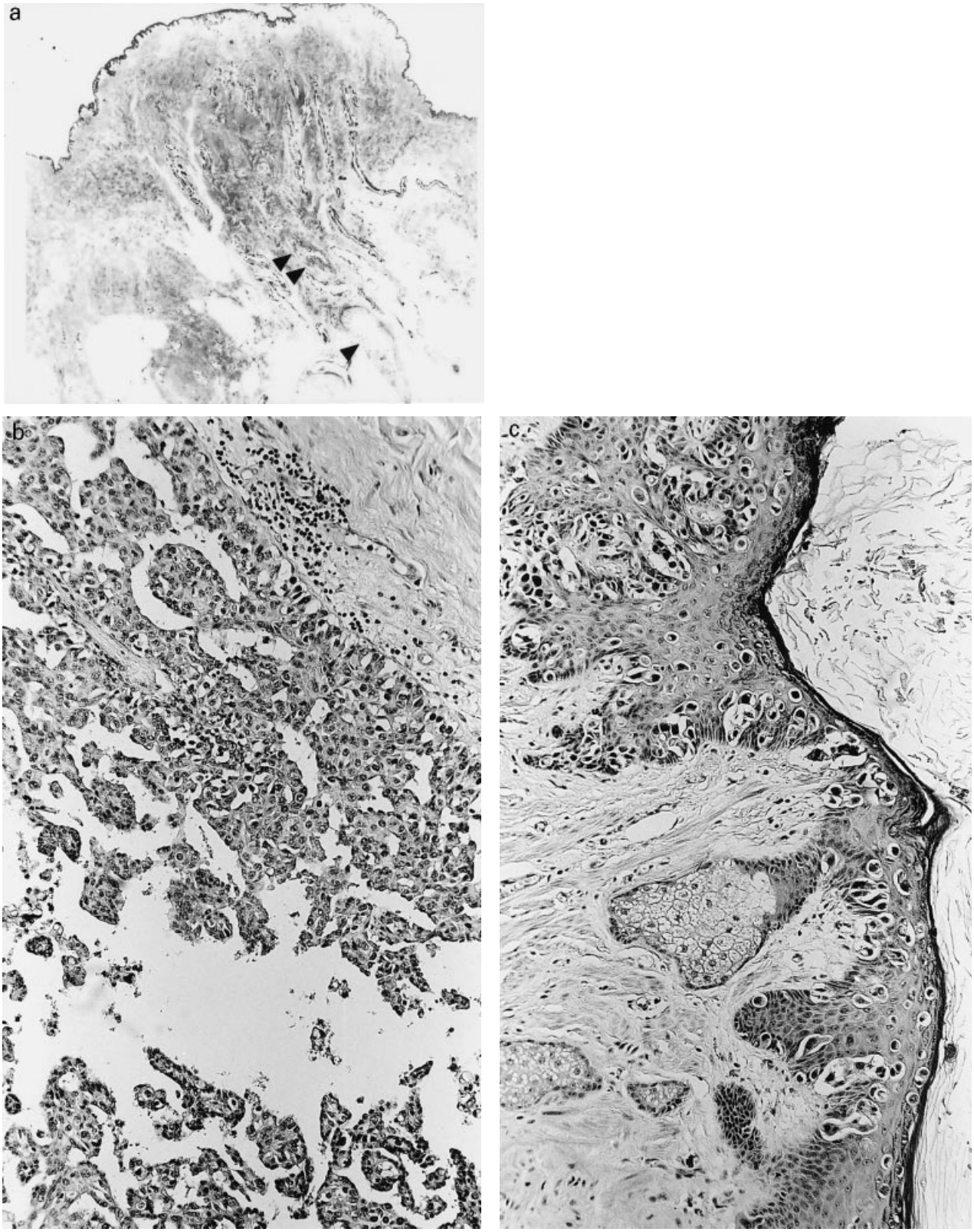

Figure 1. A case of Paget's disease involving the lactiferous duct and more distal portion of duct. **a,** Low magnification showing the DCIS involving a lactiferous duct (arrowheads). **b,** High magnification showing the DCIS (single arrowhead in Figure 1a). **c,** High magnification showing the Paget's disease.

DCIS are reviewed, and an alternative pathogenesis is proposed mechanism for the development of mammary Paget's disease. This in turn explains the similarities between mammary and extramammary Paget's cells and intraductal carcinoma cells.

Materials and methods

Twenty-eight cases of mammary Paget's disease were retrieved from the surgical pathology files at The Ottawa Hospital, Civic Campus. For all cases, in addition to sections of the deep breast tissue, the nipple was sectioned in a plane perpendicular to the skin surface. The size of each lesion was measured on the slides with the diameter recorded as the greatest dimension of the skin involved by the tumour cells. The depth was recorded as the greatest length of duct containing intraductal carcinoma or the distance from the nipple skin to the deep margin of the invasive carcinoma. Tissue blocks containing areas of epidermis with Paget's cells in the peripheral epidermal margins of the lesions in 10 randomly chosen cases of Paget's disease with skin lesions measuring more than 10 mm were serially sectioned. Four-micron thick sections of formalin-fixed and paraffin-embedded tissue were submitted for haematoxylin–phloxin–saffron (HPS) staining and immunostaining. Immunostaining was performed with following antisera: cytokeratin 7 (CK7) (Dako, Glostrup, Denmark, monoclonal, dilution 1:100), Her2/neu (Pharmagen, Ontario, Canada, dilution 1:20) in all 28 cases, 10 cases of squamous cell carcinoma and four cases of extramammary Paget's disease (of the vulva and anus).

Results

In all 28 study cases the DCIS involved the proximal portion (near the skin) of the lactiferous ducts and adjacent epidermis, as well as more distal portions of duct. The intraductal carcinoma was usually of high grade and of comedo or solid types (Figure 1a–c).

In the epidermis, there were atypical cells with abundant cytoplasm and hyperchromatic nuclei. The

Table 1. Pathological and clinical follow-up

Groups of lesion (number of cases)	Depth in mm (mean)	Diameter in mm (mean)	Immunostaining			
			+ Her2/neu		+ CK7	
			Paget's	DCIS	Paget's	DCIS
Superficial lesions						
Without invasive carcinoma (5)	3–15 (8)	10–20 (15)	5	5	5	5
With invasive carcinoma in the nipple (1)	7	15	1	1	1	1
With a separate area of invasive and intraductal carcinoma in deep tissue (2)*	10 (10)	10 and 15	2	2	2	2
Deep lesions						
Without invasive carcinoma (12)†	15–40 (29)	1–15 (10)	11	11	11	11
With invasive carcinoma (8)‡	15–40 (31)	10–15 (12)	6	6	6	6
Total (28)	3–40 (25)	5–15 (12)	25	25	25	25

* The cross-sections of the nipple at the base contained no intraductal carcinoma. The areas of carcinoma (intraductal carcinoma in one case and intraductal and invasive carcinoma in the other case) in the deep breast tissue in both cases were in the direction of the longest lactiferous ducts having intraductal carcinoma. †A single case of invasive lobular carcinoma associated with low grade DC1S and Paget's disease. ‡ Two cases were associated DCIS with skip areas.

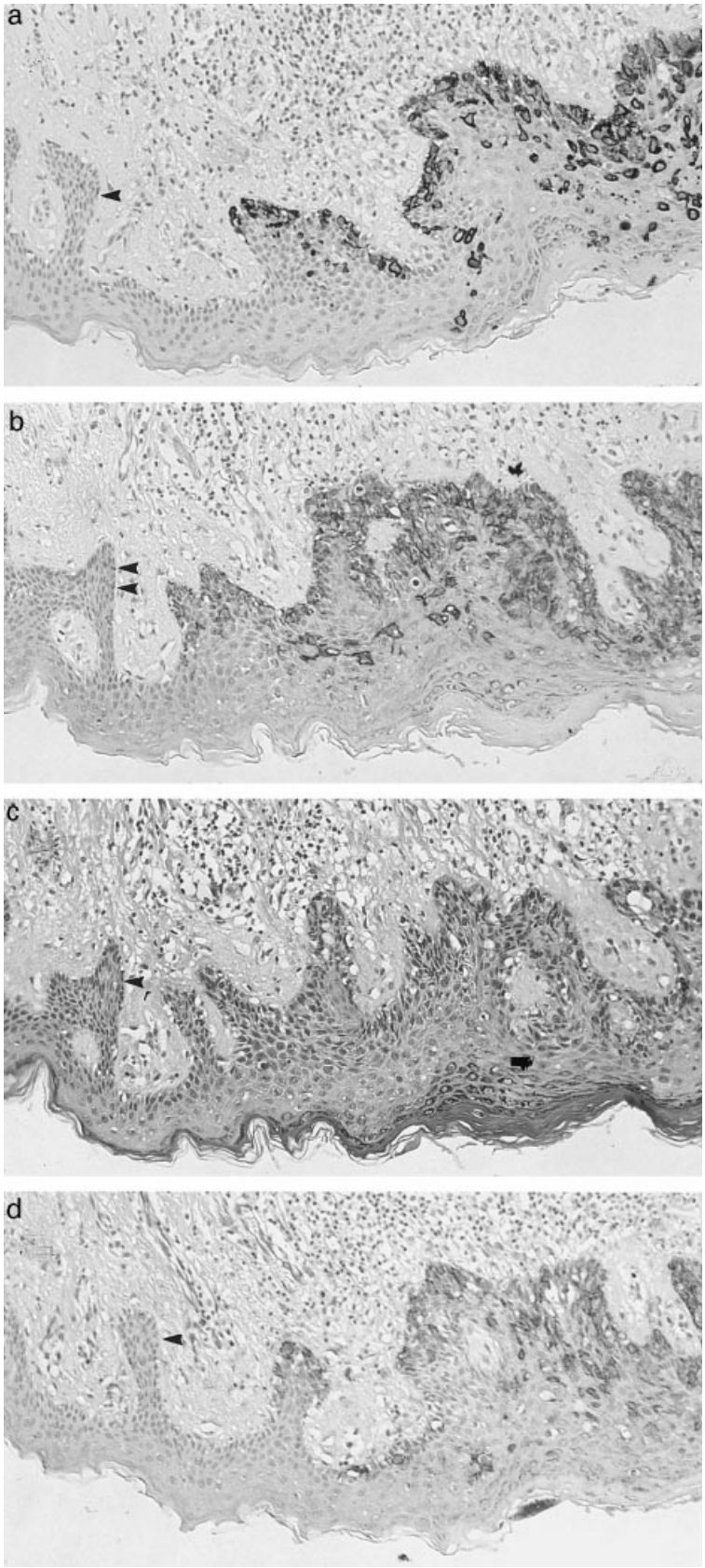

Figure 2. Serial sections (in respective order **a–d**) of an area of nipple skin with Paget's disease showing Paget's cells in a large number in the upper epidermis with only scattered tumour cells in the upper epidermis in the centre of the lesion and scattered isolated tumour cells in the basal cell layer at the periphery of the lesion. (The arrowheads in the serial section indicate the same area in the tissue.) **a**, Immunostaining with cytokeratin 7. Note the absence of the immunostaining in the area indicated by arrowheads. **b**,**e**, Immunostaining with Her2/neu. Note the presence of immunoreactive cells in the basal cell layer (arrowheads) (**e**, Higher magnification of the area indicated by arrows in B.) **c**,**f**, HPS staining showing the Her2/neu positive cells (arrowheads) in the basal cell layer (**f**, Higher magnification of the area indicated by arrow in **b**.) **d**, Immunostaining with Her2/neu. Note the absence of the immunostaining in the area indicated by arrowheads.

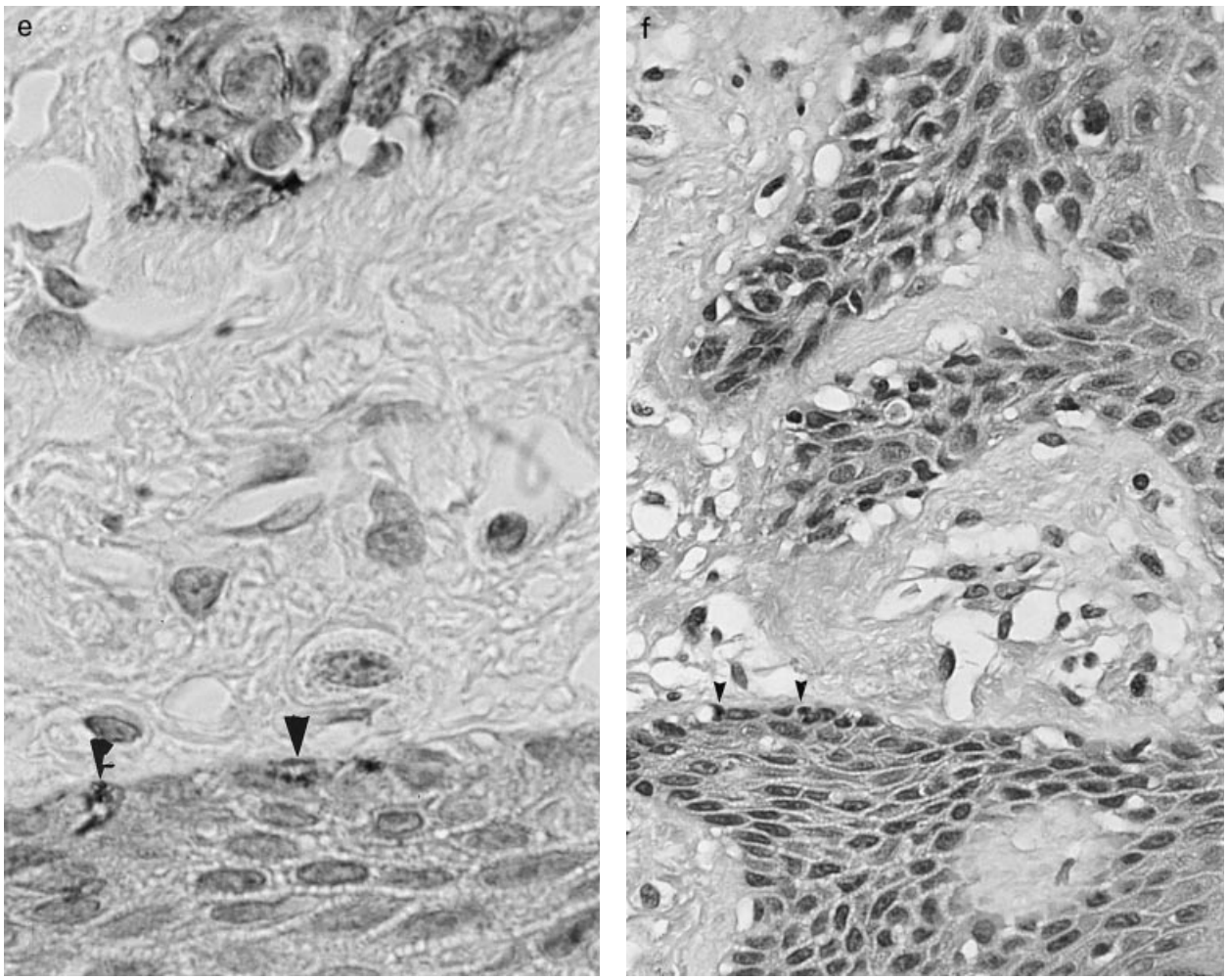

Figure 2. *Continued.*

cell size and nuclear size displayed a range of changes varying from sizes of basal cells to those of the underlying breast tumour cells. The cytoplasm of tumour cells also displayed a range of changes varying from clear to thick cytoplasm. Clear tumour cells were usually large and had a mucin-like appearance. The tumour cells were seen predominantly at the lower level, however, scattered cells in the upper portions of the epidermis were also present (Figures 1c, 2a–d, 3a,b). At the periphery of the epidermal component with serial sectioning in ten cases, there were foci of single or groups of small atypical cells at the basal cell layer. These were separated from Paget's cells towards the centre of the lesion by normal basal cells (Figures 2a–f).

Examination of sections of all 28 cases in this study revealed the DCIS in the deep breast tissue displaying 'skip lesions' in four cases (Table 1). Skip lesions were characterized by foci of DCIS in the same duct separated by a long portion of duct without significant epithelial hyperplasia (Figure 4a,b).

Immunostaining with CK7 and Her2/neu showed a strong reactivity in almost all atypical cells in the epidermis (Figures 2a–f and 3a,b) and tumour cells in the DCIS component in 25 cases. At the periphery of the lesion involving the epidermis, there were single cells or clusters of cells at the basal cell layer which displayed positive reactivity (Figures 2a–f and 3a,b). Reactivity with CK7 and Her2/neu was negative for all squamous cell carcinoma and for cases with extramammary Paget's disease.

Electron microscopy examination of the epidermis in all three, cases of Paget's disease revealed nests of tumour cells characterized by the abundance of rough endoplasmic reticulum, the rarity of intermediate

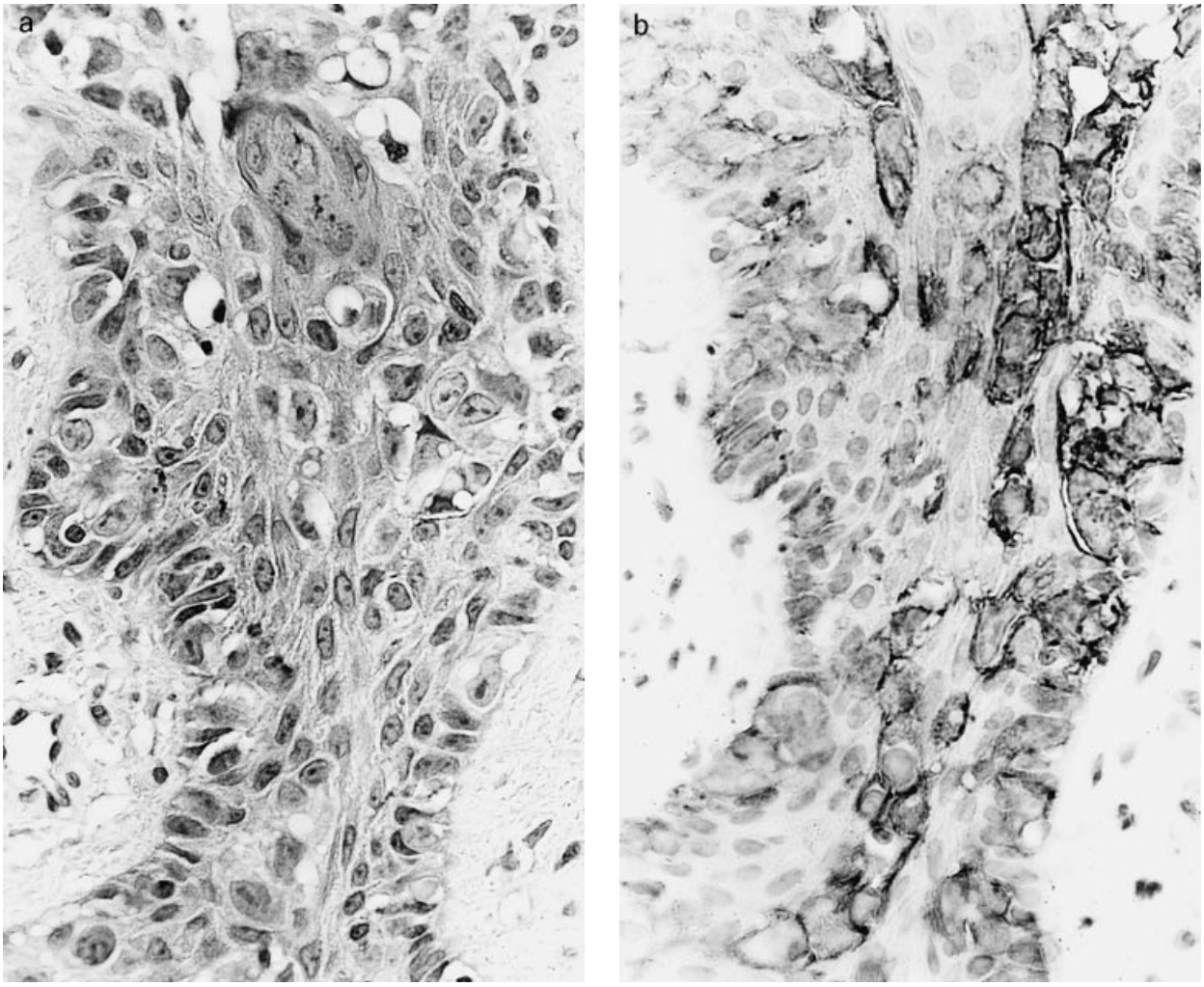

Figure 3. Serial sections of a rete peg of the nipple skin with Paget's disease. (The arrows in the serial section indicate the same area in the tissue). **a**, HPS staining. **b**, Immunostaining with Her2/neu showing positive reactivity in tumour cells having features of basal cells with HPS staining.

filaments and the absence of tonofilament bundles. A varying number of desmosomes were identified between tumour cells and squamous cells whereas desmosome-like structures were often seen between tumour cells (Figure 5).

Discussion

Mammary Paget's disease is often believed to develop as a result of tumour expansion and/or tumour cell migration from an underlying DCIS.[7,11,12] This theory of pathogenesis of mammary Paget's disease is based on: (a) the frequent association with an underlying DCIS showing a connection between the intraductal and intraepidermal components, as evidenced in this study; (b) the similarity of histopathology and immunohistochemistry between atypical cells in the epidermis and those in the underlying breast carcinoma;[1–3] and (c) the possible motility of the malignant cells in response to chemotactic factors secreted by the keratinocytes.[13,14] However, electron microscopy in this and in previous studies[15] have demonstrated the presence of desmosomes or desmosome-like structures between Paget's cells and adjacent keratinocytes. Although cell junctions are labile structures, it is unlikely that mobile and malignant cells can compress and push away surrounding cells moving between these cells and induce 'reactive' cell junctions with adjacent benign keratinocytes. Furthermore, this mode of tumour spread in a radial direction could not be

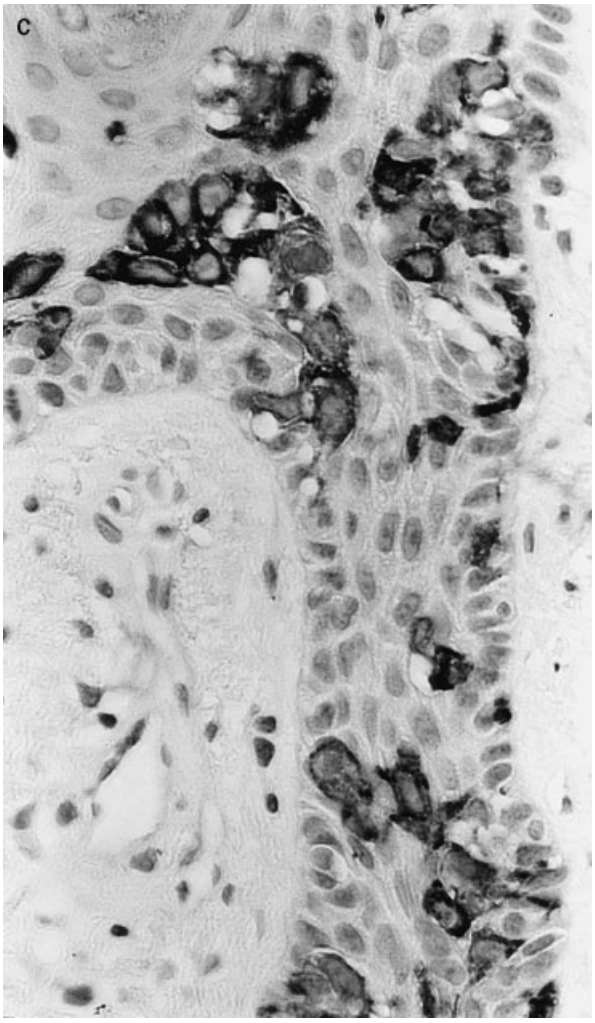

Figure 3. *Continued.*

accounted for by the predominance of the atypical cells in the lower layer of the epidermis with gradual enlargement of tumour cell size in the upper layers of the epidermis.

As a result of the findings in this study, I believe that one of the mechanisms of tumour spread from the underlying DCIS into the epidermis is a combination of: (a) neoplastic transformation of the pluripotent epidermal basal cells and (b) field effect. The evidence for neoplastic transformation of epidermal basal cells comprises:

(a) The presence of a spectrum of changes displayed by atypical cells in the epidermis and in some cases, an appearance simulating a squamous cell car-cinoma in situ.

(b) The prevalent location of Paget's cells in the lower portion of the epidermis suggests the origin of these cells from the basal cell layer (that have the pluripotential of cellular differentiation). Autoradiograph after incorporation of tritiated thymidine detected neoplastic cells in the deepest layers of the epidermis of the skin of mammary Paget's disease.[16]

(c) The presence of Her2-reactive basal-like cells in groups or in a single cell pattern in the basal cell layer of the epidermis.

The increase in nuclear size and in the degree of nuclear atypia of Paget's cells in basal layer as compared with those in the upper layer of the epidermis were characteristic of this pathological entity. Metastatic breast carcinoma in lymph nodes or structures other than the epidermis do not display this spectrum and extent of changes of neoplastic cells. Even in the intraductal carcinoma of the breast, such a spectrum of changes is not seen. Therefore, it is likely that this characteristic spectrum is attributed to the inherent properties of the constituent cells of the epidermis rather than to the 'environment' of the different levels of the epidermis.

This study also demonstrated that reactivity with CK7 and Her2/neu although present in a variety of pathological conditions was specific and sensitive for Paget's cells in the setting in this study. Furthermore, the changes in the epidermis with isolated atypical Her2-positive cells in the basal cell layer, the skip lesions of DCIS and the similarities between Paget's cells and DCIS cells are likely manifestations of the field effect. Cells in this field effect are believed to belong to the same type to tissue (at least of the same embryonic origin as compared to the adjacent tissue), to be susceptible to oncogenic alteration and to be predisposed to the same factors that induce genetic alterations. These factors include heredity, hormones and possibly mutagenic factors released from adjacent tumour cells. Particularly, this genetic induction is well known in embryonic development in which a tissue induces the other adjacent tissue to transform.[17] The transformed tissue bears some similar features of the inducing tissue, as demonstrated in the development of the prostate,[18] the pathogenesis of endometriosis[19] and the development and pathological conditions of other organs.[20–22]

Cancer cells have been demonstrated to induce a neoplastic 'horizontal' transformation of adjacent stromal cells into malignant.[23–27] This mechanism of malignant progression by 'recruiting' normal cells has been proposed previously.[25] Because oncogenesis operates on proliferating cells, this field effect most likely affects progenitors of ductal cells in the duct and progenitors of 'clear cells' (basal cells) in the epidermis.

It is known that 'Toker's clear cells' are occasionally identified in the epidermis' and have the histochemical and immunohistochemical properties similar to those of

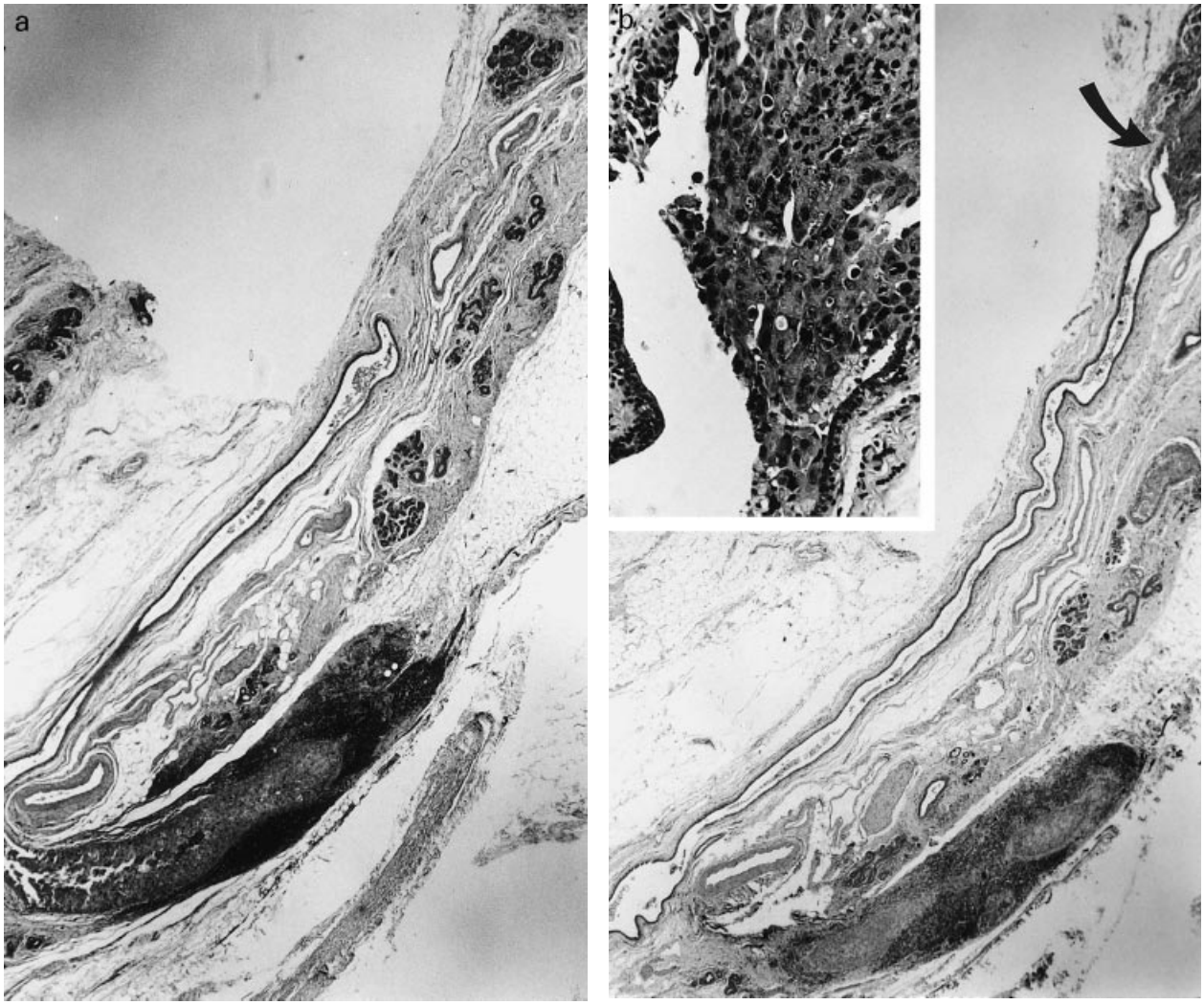

Figure 4. a,**b**, Representative serial sections of a duct in a case of Paget's disease with intraductal carcinoma involving a duct with a long portion of duct free of carcinoma. Inset in **a**: High magnification of portions of duct with tumour edge.

ductal cells.[28–30] They are believed to represent the normal counterpart of Paget's cells.[28–30] Unlike lobular carcinoma in situ often involving lobules and affecting adjacent small ducts, intraductal carcinoma and especially high grade carcinoma tend to affect large ducts. Due to the anatomical location, Toker's clear cells fall into the field effect with high-grade DCIS.

In conclusion, the findings in this study suggest that Paget's cells share features of both squamous cells and underlying DCIS cells. The possible explanation for this hybrid feature of Paget's cells is the malignant transformation of basal cells in the epidermis secondary to field effects with the implication of the potential of cancer cells to induce malignant transformation of 'host cells'. It is realized that this study was limited to the morphological and immunohistochemical changes and further studies on genetic alterations are necessary to support the possibility of field effect with 'horizontal' induction by cancer cells.

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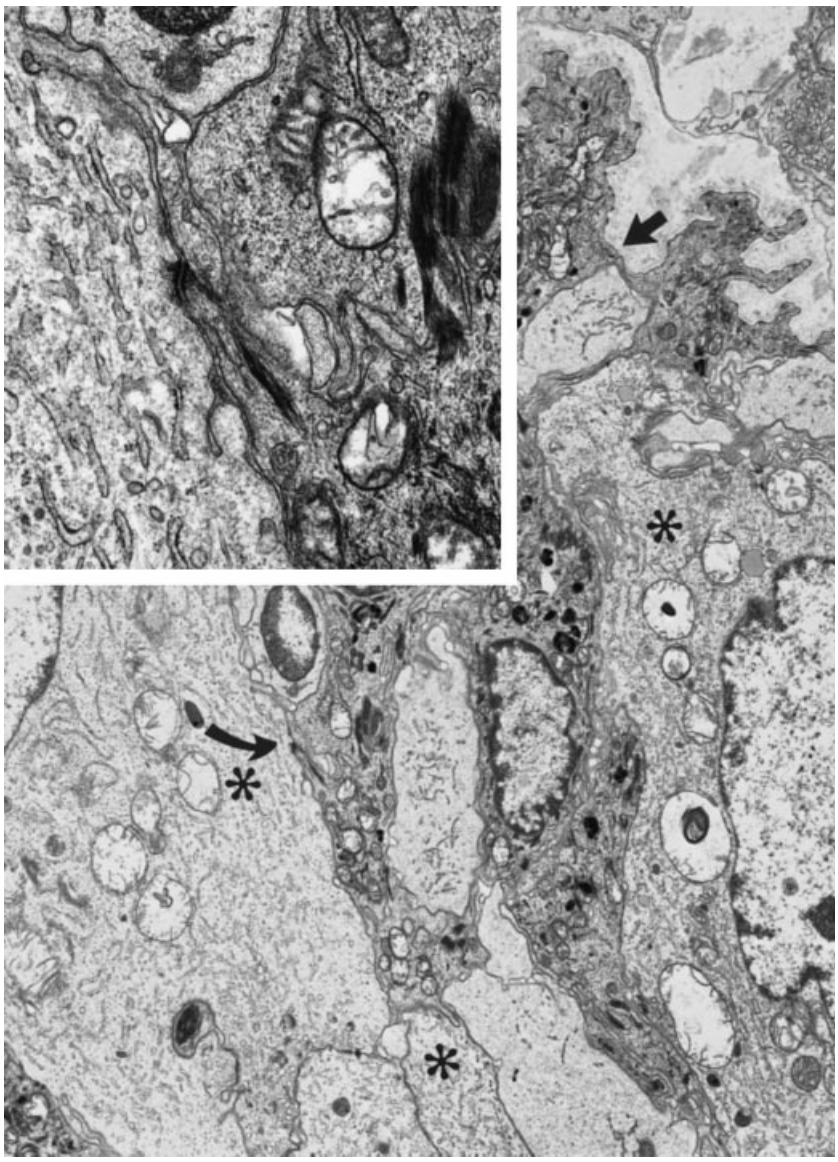

Figure 5. Ultrastructural appearance of the lower portion of the epidermis containing Paget's cells (asterisks) characterized by the clear cytoplasm with an abundance of rough endoplasmic reticulum, in contrast with squamous cells with dark cytoplasm and tonofilaments. Note the basement membrane (arrow) underlying the basal cells. Inset: High magnification of an area indicated by a curved arrow in **a** showing a desmosome connecting a Paget's cell with an adjacent squamous cell.

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